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Pain by association? Experimental modulation of human pain thresholds using classical conditioning.

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Abstract

A classical conditioning framework is often used for clinical reasoning about pain that persists after tissue healing. However, experimental studies demonstrating classically conditioned pain in humans are lacking. The current study tested whether non-nociceptive somatosensory stimuli can come to modulate pain threshold after being paired with painful nociceptive stimuli in healthy humans. We used a differential simultaneous conditioning paradigm in which one non-painful vibrotactile conditioned stimulus (CS+) was simultaneously paired with an unconditioned painful laser stimulus (US), while another vibrotactile stimulus (CS-) was paired with a non-painful laser stimulus. After acquisition, at-pain-threshold laser stimuli were delivered simultaneously with a CS+ or CS- vibrotactile stimulus. The primary outcome was the percentage of at-threshold laser stimuli that were reported as painful. The results were as expected: after conditioning, at-threshold laser trials paired with the CS+ were reported as painful more often, as more intense, and as more unpleasant than those paired with the CS-. This study provides new evidence that pain thresholds can be modulated via classical conditioning, even when the stimulus used to test the threshold can not be anticipated. As such, it lays a critical foundation for further investigations of classical conditioning as a possible driver of persistent pain.

Perspective

This study provides new evidence that human pain thresholds can be influenced by non-nociceptive somatosensory stimuli, via a classical conditioning effect. As such, it lays a critical foundation for further investigations of classical conditioning as a possible driver of persistent pain.

Keywords

Pain; classical conditioning; allodynia; pain threshold; Pavlovian conditioning

Introduction

The persistence of pain after tissue healing is poorly understood[23], but a classical conditioning framework is commonly used to discuss it, e.g. [2, 19, 24, 41]. Classical conditioning is a form of associative learning in which a neutral (conditioned) stimulus acquires motivational features after being paired with another, biologically evocative (unconditioned) stimulus that inherently elicits an unconditioned response, and thus elicits similar (conditioned) responses, even in the absence of the unconditioned stimulus[26]. When applied to pain, the classical conditioning framework predicts that a non-noxious stimulus may come to elicit a pain response after being paired with a noxious stimulus that is inherently perceived as painful. For example, a person who repeatedly experiences pain—driven by nociception—when bending forward after a back injury may continue to experience pain on bending forward, even after the injured tissues have healed and nociception has ceased or returned to baseline levels – that is, the movement is no longer truly harmful. The classical conditioning explanation for this persistent pain would be that repeated pairing of non-noxious with noxious stimuli in the acute phase has rendered the non-noxious stimuli capable of eliciting a similar response—pain.

There is much evidence to support that *fear of pain* can be acquired by classical conditioning[34]. This fear is thought to drive the progressive avoidance of activities that leads to disability in people with chronic pain[39]. In fact, the knowledge that fear can be a classically conditioned response has laid the foundation for new treatments that directly target the cause of avoidance and disability in people with chronic pain[3, 12, 35]. Critically, such treatments do not primarily aim to change pain, but to change fear and avoidance behaviour.

The idea that *pain itself* could be a classically conditioned response seems intuitive, and most practising healthcare clinicians endorse it[17]. The stimulus pairing that drives classical conditioning is clearly mimicked by the pairing of noxious and non-noxious input in the acute phase of a tissue

injury, and classical conditioning is capable of eliciting hyperalgesia[16]. However, contrary to popular clinical views[17], there is very limited evidence as to whether pain itself can also be classically conditioned[16]. Our recent systematic review revealed only three studies that could shed light on this issue, and only one noted a conditioned shift in pain threshold. The change in pain threshold was thought to have occurred secondary to a conditioned change in arousal and valence, because that study used both emotive CSs and forward timing[38]. One critical gap is whether or not classical conditioning can endow non-noxious stimuli with the ability to induce allodynia, in which pain is elicited at a lower intensity of nociceptive stimulation than would normally be required for pain—and whether such an effect can exist without the anticipatory period that occurs with forward timing of stimuli.

We aimed to test whether classical conditioning could modulate pain thresholds to laser stimuli. We used a simultaneous conditioning paradigm with vibrotactile stimuli in two different anatomical locations as conditioning stimuli (CSs), of which one (CS+) was paired with painful laser stimulation (unconditional stimulus, $US_{High(H)}$), and the other (CS-) with non-painful laser stimulation ($US_{Low(L)}$). We then tested for a classically conditioned shift in pain threshold by delivering at-pain-threshold laser stimuli (US_T) simultaneously with the CS+ and the CS-, and comparing the proportions of trials reported as painful with each CS. We hypothesised that, after the conditioning procedure, the compound CS+/US_T trials would be reported as painful more often than the compound CS-/US_T trials.

Methods

Subjects

We recruited healthy adult participants using flyers and word of mouth. Study information was provided electronically and verbally, or in print. Participants were screened on the telephone or via email, and again on arrival for testing. Participants were compensated at AUD20/hour (with a

maximum of $60) for inconvenience and travel costs. Written informed consent was obtained. All procedures conformed to the Helsinki Declaration and were approved by the institutional ethics committee.

Inclusion criteria were pain-free status, age over 18, and ability to consent autonomously. Screening exclusion criteria were: pain at the time of testing, use of analgesic medication on the day of testing, use of medication that could alter skin sensitivity or healing, skin condition inadequate to tolerate laser application without damage, a history of chronic pain (defined as pain every day for 3 months or longer [8]), sensation problems, diagnosed peripheral vascular disease, diabetes mellitus, neurological problems, or a previous or current psychiatric diagnosis.

Additional exclusion criteria were implemented mid-procedure. These were: (1) rapid reddening of skin after laser stimuli, (2) pain threshold too low (an equipment limitation), (3) less than 50% of US_H laser stimuli rated as painful during the acquisition phases, and (4) 50% or more of US_L laser stimuli rated as painful during the acquisition phases (see below for details). Participants who were excluded according to these criteria were not considered further. All excluded participants were replaced.

We were unable to compute a robust estimate of required sample size *a priori* because no previous research exists to provide adequate information for this calculation. According to an *a priori* decision, we used effect size data from Wiech et al [37] to provide an approximate estimate of the sample size needed, and then updated the estimation using the variance from our own data at n=6 and again at n=10. Sample size estimations were computed using G*POWER (version 3.1.9.2, Heinrich Heine Universität Düsseldorf, Germany)[13], and performed by an independent statistician who did not run a full analysis of the data. In accordance with the *a priori* plan, we ceased data

collection once we reached the sample size (n=16) estimated by the calculation that had been done, by the statistician, using the data from the first 10 participants.

Stimuli

Laser stimuli were used as USs. They were delivered using an Nd:YAP laser stimulator (Stimul 1340, Deka®, Italy, pulse duration 6ms, spot diameter 4mm). At low intensities this stimulus may not be felt at all or may elicit a warm sensation, thought to reflect activation of C-fibre nociceptors. At higher intensities this stimulus may also elicit an additional pinprick sensation, thought to reflect activation of A -fibre nociceptors [29]. The location of the laser stimulus was shifted slightly between trials to prevent skin damage.

Vibrotactile stimuli (duration 500ms) at two different locations were used as CSs, and were delivered using adapted mobile phone vibrators (tactors) that were manually controlled using a program developed in house, via LabVIEW™. The tactors were fixed to the skin of the back with double-sided tape (see Figure 1) and were set to vibrate at a clearly perceptible, non-painful intensity. Participants were allocated to receive vibration at one site (cephalad or caudad) as the CS+, and at the other site as the CS-. Allocations of location and tactor were counterbalanced across participants, and assigned according to a pre-randomised order. For each compound trial (involving vibrotactile and laser stimulation), the onset of the two stimuli was simultaneous. The term 'stimulus package' was used for single-modality trials and dual-modality trials. We also provided vibrotactile stimulation at a neutral location during the threshold test (see below). For this, we used a third tactor, in a third location, with the same stimulus parameters as for the CSs (see Figure 1).

Outcomes and measures

Manipulation checks

We used two manipulation checks to test the conditioning procedure. Participants had become familiar with naming each CS according to whether it was closer to the head or to the feet.

Therefore, for the US expectancy ratings, participants responded to the question "*To what extent do you expect the stimulus package to be painful (rather than non-painful) if the stimulus package includes the head/feet [separate questions] vibration*?" on a 0-10 NRS with anchors of 0 = "I do not expect that it will feel painful" and 10 = "I fully expect that it will feel painful". See Supplementary File 1 for the exact text used. These expectancy ratings were obtained between phases, yielding expectancy rating data at three time points: 'baseline' (before acquisition), 'post-acquisition' (after acquisition) and 'post-test' (after the test phase). The second manipulation check was a comparison between phasic electrodermal responses (EDRs) to CS+ trials and CS- trials in the acquisition phase, to test whether the two types of trial evoked different levels of arousal. Electrodermal activity was recorded using two self-adhesive Ag/AgCl resting ECG electrodes (Tyco Healthcare Group®, Mansfield, MA, USA) attached to the middle and distal phalanges of the index and middle fingers of the left hand and connected to a Galvanic Skin Response device (780273 GSR, Frederiksen®,Denmark). The signal was recorded using Scan 4.5 software (Neuroscan, Compumedics®, Australia), at 250Hz (samples per second) with a low pass of 100Hz. Events were labelled using Curry 7 software (Neuroscan, Compumedics®, Australia). Ledalab's Continuous Decomposition Analysis [5] was used to identify and quantify phasic EDRs with a minimum amplitude deflection of 0.01µS within a response window of 1-5 seconds after each event. The average phasic driver within the response window was recorded for each event, and these were compared.

Intensity of stimulus package

We wanted to know whether each trial was perceived as painful or non-painful, and how intense that painful or non-painful experience was. Participants were therefore instructed to report on their experience of each 'stimulus package' using the Fifty Either Side of Threshold Numerical Rating Scale (FESTNRS)[15], which has anchors of "no sensation" (-50), "the exact point at which what you feel transitions to pain" (0), and "most intense pain you can imagine" (+50). A visual version of the FESTNRS was used to reinforce the meaning of the anchors by showing the range of -50 to 0 as "non-

painful" and the range of 0 to +50 as "painful". Participants were allowed to use any number within the range of the scale, except 0. They were coached on using this scale, and instructed to make an initial decision about whether the trial was painful or non-painful. They then used the appropriate side of the scale to rate the intensity of the experience. The primary outcome for this study was the number of at-threshold trials rated as painful during the test phase. The FESTNRS allowed us to extract information in this binary form (painful or non-painful, as the primary outcome) without losing the sensitivity that an interval NRS provides. Participants were instructed to give their reports only 2-5 seconds after stimulus onset, so as to prevent speech from interfering with the measurement of electrodermal activity.

Valence of stimulus package

We also wanted to know whether each trial was experienced as pleasant or unpleasant, and the intensity of that experience. Participants rated this on a version of the FESTNRS that had been adapted to measure valence by using anchors of "extremely unpleasant" (-50), "neutral" (0), and "extremely pleasant" (+50). Again, a visual scale was used to reinforce the meaning of the anchors by showing the range of -50 to 0 as "unpleasant" and the range of 0 to +50 as "pleasant". Participants were allowed to use any number within the range of this scale, now including 0. Participants were coached to make an initial decision about whether the trial was pleasant or unpleasant, and then choose a number. Participants were encouraged to use the two scales (for painfulness and valence) independently, and not to try to match up the ratings with one another. We wanted to explore the influence that psychological variables might have on the primary outcome. We therefore measured depression, anxiety and stress, positive and negative affect, and the extent to which participants habitually engage in catastrophic thinking about pain.

Individual differences

We were also interested in the influence that individual differences in affective state might have on the primary outcome. We therefore measured depression, anxiety and stress, positive and negative

affect, and the extent to which participants habitually engage in catastrophic thinking about pain. Depression, anxiety and stress were measured with the Depression Anxiety Stress Scale (DASS) [14]. The DASS subscales have shown high correlation with other scales measuring similar constructs and good internal consistency in clinical and non-clinical groups [1, 20]. Positive and Negative Affect at the time (i.e. state) were measured with Positive and Negative Affect Schedule (PANAS) [36], the subscales of which have shown good internal consistency (Cronbach's α for PA and NA scales were .89 and .85 respectively) and construct validity in a general adult population [9]. The extent to which participants habitually engage in catastrophic thinking about pain was measured with the Pain Catastrophising Scale (PCS) [33]. When tested in a community samples, the PCS has shown good internal consistency (Cronbach's α = .87) and test-retest reliability (r = .70-.75) [25, 33].

Perceptions

We used post-experiment questions to explore participants' perceptions of (1) the aim of the study, so as to assess the effectiveness of blinding, (2) the differences between vibrations at the two locations, in order to detect any subtle differences in vibration quality that could have contributed to learning, (3) the timing of the laser stimuli relative to the vibratory stimuli, (4) the relationship between the vibratory stimuli and how painful the laser stimulus was, in order to identify conscious awareness of pairing, and (5) whether or not participants had been able to *predict* the intensity of the laser stimulus on the basis of which CS was received, at the time of each trial (see Supplementary File 2).

Experimental procedure

This study followed a within-subject design and required participants to attend two testing sessions, starting at the same time on two consecutive days. This reduced variance by increasing the number of trials without causing skin damage. The three core phases (calibration, acquisition, test) were identical on the two days.

Preparation

Participants read through the study information sheet and had an opportunity to ask questions. They were given no precise information about the purpose of the study, so as to ensure blinding. Consent forms and questionnaires were completed, and the EDA electrodes were attached. Testing was performed with participants lying prone on a plinth. The hair was trimmed from the stimulation areas. Skin markings were made to delineate the areas to be used for laser stimulation and the tactor positioning. The two CS tactors were placed about 70mm to the left of the thoracolumbar spinous processes, 70mm apart. The third tactor, which was only used for the calibration phase, was fixed 15mm lateral to the lateral border of the left laser stimulation zone. The main laser stimulation zone was midway between the main tactors (see Figure 1), and measured 50x70mm. A second laser stimulation zone was delineated on the right side of the back, to be used to orientate participants to receiving and reporting on laser stimuli.

Participants wore headphones, and arbitrary, repetitive noise (Sleep Pillow app for iPhone, ClearSkyApps) was played during trials, to drown out the sound of the laser machine's foot pedal, and to guide participants not to provide ratings too soon after stimulus delivery. Participants were instructed to speak only when the sound was switched off.

A practice phase was used to familiarise participants with rating laser stimuli using the FESTNRSs. Participants received five laser stimuli of each possible intensity (in steps of 0.25J) between 1.00J and 4.00J, in random order. They rated their experience on the two NRSs. (These data are not reported here, but some are reported in [15].) If participants reported particularly high pain ratings (greater than +35) or showed marked, localised reddening of the skin during this phase, then trials of intensity greater than 3J were omitted at the discretion of the operator. After this phase, if a participant's skin was reddened, the block of skin to which stimuli had been delivered was slowly cooled for 2-3 minutes, using a refrigerated gel pack. Participants then stood and walked around during a break of 2-5 minutes.

Main procedure

1) Calibration phase

The goal of this phase was to establish the intensity of laser stimulus that matched the participant's pain threshold *when that laser stimulus was accompanied by a vibrotactile (VT) stimulus* (see Figure 2). This intensity would later become the test stimulus, US_T. We therefore delivered dual-modality trials, using both laser and a VT stimulus at the lateral tactor. The lateral tactor was used so as to provide a VT stimulus that was neutral with respect to the subsequent conditioning procedure.

The tactors were fixed to the left side of the back as described above, and participants were oriented to the locations of the tactors. We tested that they could differentiate the locations of the three tactors by randomly activating each and asking the participant to identify which one had been activated. Participants were then introduced to the calibration phase as "another practice phase" during which only the lateral tactor would be used, so that they could become accustomed to rating trials that involved both laser and VT stimulation. Dual-modality trials were delivered in an adaptive staircase procedure (Best-PEST calculator [40]). Participants rated each trial on both FESTNRSs, and the reports from the intensity FESTNRS were used to estimate the participant's pain threshold. After this phase, the lateral tactor was removed, and participants were told that they could only receive vibrations at the locations closer to the "head" or to the "feet" from that point onwards.

2) Baseline phase

Next, a baseline test of CS perception was performed. Ten vibration-only trials (5 x CS+; 5 x CS-) were delivered, and participants provided ratings for each stimulus package. This phase was followed by the first set of expectancy ratings.

3) Acquisition phase

The acquisition phase comprised 12 painful laser stimuli (US_H, approximately pain threshold + 0.75J) paired with the CS+, and 12 non-painful laser stimuli (US_L, approximately pain threshold minus 0.5J) paired with the CS-. The onset of each CS was timed to coincide with the onset of the US. These

trials were randomly ordered, using a Microsoft Excel (2013, ©Microsoft Corporation) spreadsheet, under the restriction that no more than three identical trials could be delivered in succession. The reinforcement contingency was set to 100% for the acquisition phase because we predicted that this contingency would naturally be diminished - some supra-threshold laser stimuli would be perceived as non-painful, and vice versa - due to the variable percept that is typical of Nd:YAP laser stimulation. However, a mid-procedure exclusion criterion eliminated participants who experienced ≤50% reinforcement of CSs during this phase. Participants were required to rate their experience of each trial on each FESTNRS. This phase was followed by the second set of expectancy ratings.

4) Test phase

The test phase comprised three blocks of 40 trials each, with one opportunity for a brief break as required. Each block included 10 compound CS+/US_H trials, 10 CS-/US_L trials, 5 CS+/US_T trials, 5 CS-/US_T trials, 3 CS+-only trials, 3 CS--only trials, and 4 US_T-only trials (see Figure 2). The reinforcement trials (10 compound CS+/US_H trials, 10 CS-/US_L trials) were included so as to prevent extinction. In this way we obtained 30 *paired* trials of the at-threshold laser stimulus, 15 of which were paired with the CS+ and 15 with the CS-. This phase was followed by the third set of expectancy ratings. Participants' skin was cooled if slightly reddened. Participants were informed that the session ended and asked to return the following day at the same time. Once all testing had been completed, participants were thanked for their participation, filled in an honorarium form, and left.

Simultaneous timing

We wanted to minimise the risk that time-contingent processes that are known to influence pain, such as expectation, arousal, and fear, could mediate our results. We therefore used an atypical simultaneous pairing of CS and US, rather than the more conventional delay timing. CS and US began simultaneously, but the CS (duration 500ms) ended before the US (duration 6ms). By making the stimuli begin simultaneously, we minimised the possibility that participants could predict which US they were to receive on the basis of which CS was presented.

Statistical analyses

General approach

Data were visually inspected for distribution, and tests were applied to confirm that the data met the applicable test assumptions: before t-tests, normality of the sampling distribution of the differences was checked, and before repeated measures (RM) Analysis of Variance (ANOVA), sphericity was checked. Non-parametric tests were used when appropriate. Where the assumption of sphericity was violated, adjusted values are reported, with degrees of freedom also adjusted accordingly. Following ANOVA, planned comparisons were used to investigate significant effects, and Bonferroni adjustments were applied to correct for multiple comparisons. Alpha was set at 0.05. Descriptive data are reported in Table 1.

Manipulation checks

Expectancy ratings were analysed using a 2 (Condition: CS+ or CS-) x 3 (Time: baseline (before acquisition), post-acquisition, and post-test) repeated-measures ANOVA. We anticipated no difference in expectancy to CS+ trials vs CS- trials at baseline, higher expectancy to CS+ trials than to CS- trials at post-acquisition and at post-test, and that the difference in expectancy at post-test would be smaller than that at post-acquisition.

EDR_{amp} data were not normally distributed, and were therefore compared using a one-tailed Wilcoxon signed ranks test, because we clearly hypothesised that compound CS+/US_H trials would evoke greater arousal than CS-/US_L trials in the acquisition phase.

Primary analysis

In order to test the primary hypothesis—that trials involving at-threshold laser stimuli paired with the CS+ (compound CS+/US_T trials) would be reported as painful more often than those involving the CS- (compound CS-/US_T trials)—the percentage of US_T trials rated as painful was compared across conditions (paired with CS+ versus with CS-). A 2 (Condition: CS+ v CS-) x 2 (Session: 1 vs 2) RM ANOVA was used to check for an effect of Session. In the absence of such an effect, the results for

sessions 1 and 2 were collapsed and means for the two test phases were used for all analyses thereafter. A paired t-test was used to compare the collapsed means.

Secondary analyses

Intensity and valence ratings for CS-only trials were rescaled to a 0-100 scale by adding 50 to each rating. A 2-way RM ANOVA was used to compare FESTNRS ratings of compound CS/US_T trials across Outcome (intensity vs valence) and Condition (CS+ vs CS-). We expected that CS+ trials would be rated as more intense and more unpleasant than CS- trials (i.e. we expected a statistical main effect of Condition). A separate 3-way ANOVA was used to compare FESTNRS ratings of CS-only trials across Phase (pre-acquisition session 1 vs mean of test sessions 1 and 2), Outcome (intensity vs valence) and Condition (CS+ vs CS-). We expected that CS+ trials would be rated as more intense and more unpleasant than CS- trials in the test phases only (i.e. we expected a statistical Phase x Condition interaction).

We explored the roles of contingency awareness, expectancy, DASS score, PCS score and NA score (from the PANAS) on the primary outcome. The influence of contingency awareness was explored by entering it as a covariate in the primary analysis. The remaining exploratory variables were entered into regression analyses as predictor variables, and the index of the classical conditioning effect was used as a dependent variable in each analysis. The index of the classical conditioning effect was computed by dividing the percentage of US_T trials rated as painful when paired with the CS+ by the same figure for US_T trials paired with the CS-. A single value for expectancy was also computed for each participant by subtracting the expectancy rating for CS+ trials from that for CS- trials at post-acquisition questioning and post-test questioning, and taking a mean of the result at these two time points. No corrections were made for these exploratory analyses.

Results

Twenty-five participants were recruited. Nine were excluded: in four participants, pain threshold was too low for our equipment, two participants reported US_L as painful in more than 50% of acquisition trials, one participant reported having back pain during testing, one withdrew because of dislike for the stimulation, and one participant was removed because of excessive movement during the procedure and failure to follow instructions. The final sample of 16 participants included nine females and seven males. See Table 1 for descriptive data on these participants, and Table 2 for summary data.

Blinding and contingency awareness

Fifteen of the 16 participants were unable to guess the purpose of the study despite thorough post-experiment questioning. One participant lost naïveté to the study question during the experiment. Eleven of the 16 participants identified that CS+ trials had tended to be more painful. We deemed them to be contingency aware.

Manipulation checks

As anticipated, differences in expectancy ratings were smallest at baseline, increased to post-acquisition, and diminished slightly to post-test. The ANOVA showed main effects of Time, $F(1.336,20.042)=10.107$, $p=.003$, $\eta_p^2=.403$, Condition, $F(1,15,)=22.542$, $p<.001$, $\eta_p^2=.600$, and a significant Time x Condition interaction, $F(1.309,19.638)=8.403$, $p=.006$, $\eta_p^2=.359$. Planned comparisons showed that expectancies changed between baseline and post-acquisition, $t(1,15)=14.753$,$p=.002$, but not between post-acquisition and post-test, $t(1,15)=1.141$,$p=.302$. Electrodermal responses were significantly greater in response to CS+ trials (median=38588.24, min=6537.68, max=252517.70) than to CS- trials (Median=29094.28, min=6709.73, max=56709.47) during the acquisition phase, $z=-2.74$, $p=.003$, $r=-.69$.

Primary analysis

Figure 3 shows the percentage of compound CS/US_T trials rated as painful in each block of the test phase, by condition. Pooled data from all three blocks showed that the compound CS+/US_T trials were rated as painful a mean of 53.75% (SD=22.51) of the time, which was more often than the 46.67% (SD=21.74) for the compound CS-/US_T trials, $t(15)=2.341$,$p=.033$,$r=.52$. Examination of Figure 3 suggests that the effect diminished across the three blocks of the test phase. However, additional analyses did not reveal a significant effect of the block, $F(2,30)=0.212$, $p=.770$, or of the block x condition interaction, $F(2,30)=1.575$, $p=.224$.

Secondary analyses

The analysis of FESTNRS ratings of compound CS/US_T trials in the test phase showed that CS+ trials were rated as more intense and more unpleasant than CS- trials (main effect of Condition,

F(1,15)=7.594, p=.015, η_p^2=.336). This difference between ratings of compound CS+/US_T trials and compound CS-/US_T trials was greater for intensity ratings than for valence ratings (interaction effect of Outcome x Condition, F(1,15)=6.013, p=.027, η_p^2=.286).

The analysis of FESTNRS ratings of CS+-only trials versus CS--only trials from the baseline and test phases showed that all CS-only trials were rated as more intense and more unpleasant in the test phase than in the baseline phase (main effect of Phase, F(1,15)=4.880, p=.043, η_p^2=.425). There was no difference in ratings of CS+-only trials compared to CS--only trials (no effect of Condition, p=.860, and no Phase x Condition interaction, p=.523).

There was no effect of contingency awareness on the primary outcome, nor was there a significant relationship between the classical conditioning effect and reported expectancy, contingency awareness, DASS score, PCS score or NA score (all p-values>.05).

Discussion

We tested whether simultaneous classical conditioning can modulate pain thresholds to laser stimuli. At-threshold laser stimuli were experienced as more intense, more unpleasant, and as painful more often, when paired with a vibrotactile stimulus that had previously been associated with a painful laser stimulus (CS+) than when paired with another vibrotactile stimulus that had been paired with a non-painful laser stimulus (CS-). In contrast, intensity and valence of the CS+ and CS- alone were not differentially affected.

Our principal finding corroborates a previous demonstration of decreased pain threshold to a test stimulus presented shortly after a fearful facial expression (as CS+) that had previously preceded a painful US several times[38]. That study's effect was attributed to the CS+ causing anticipation of the

US, causing a change in valence and/or arousal, and thereby lowering pain threshold. In contrast, this study eliminated the opportunity to anticipate the US and therefore contributes new knowledge that classical conditioning can influence pain thresholds even when the stimulus can not be anticipated. Our design strongly suggests that delay-dependent changes in arousal or valence are unlikely to have influenced participants' perceptions of the stimulus trials.

This simultaneous presentation of CS and US is an unusual feature, because a predictive role for the CS is commonly thought necessary for conditioned responding (for review, see[32]). However, simultaneous pairing sometimes has greater potency than forward pairing[4, 20, 30] – a potency that is often underestimated due to the exclusive measurement of behavioural responses that are anticipatory or predictive in nature. In humans, it is possible to measure retrospective reporting of an experience in order to infer non-predictive associative learning. Accordingly, we used simultaneous pairing, which better imitates the pairing of noxious and non-noxious stimuli in a clinical episode of pain than forward pairing.

Whereas this study found a classically conditioned shift in pain threshold, a previous study by our group found no such effect[18] - simultaneous pairing of a vibrotactile CS with a peaked painful heat US did not differentially shift pain threshold measured using a slowly ramping heat stimulus. However, that study used a different thermal stimulus for testing from the type used as the US, which may have hampered the transfer of associative learning from acquisition to test phase. Additionally, only four of the 34 participants in that study reported being aware of the CS-US contingency, which may reflect a failure to pay attention, that the stimuli used or the experimental context may not have been sufficiently salient to drive learning, or both. In contrast, expectancy and contingency awareness data showed that most participants in the current study were aware of the CS+-US_H relationship (although exploratory analyses showed that neither factor influenced the primary outcome). This is interesting in light of the dominant view that contingency awareness is

necessary for classical conditioning in humans. Indeed, evidence for an automatic, non-conscious associative learning mechanism is tenuous, while the evidence supporting a conscious, propositional learning model is more robust[22]. We found propositional knowledge, expressed here in the form of contingency awareness, to have no significant influence on the associative learning effect. This exploratory analysis might have been underpowered and that most of our participants were aware of the contingencies means this finding should be interpreted with caution.

The differential conditioned modulation of pain thresholds in this study seems, at first, to be an evaluative conditioning effect driven by a differential change in valence of the CSs[11]. However, the data do not all fit this account neatly. Trials of the CS+ alone were no less pleasant than trials of the CS- alone. In contrast, compound CS+/US_T trials were more intense, more unpleasant, and more likely to be painful than compound CS-/US_T trials. The nature of the CS clearly modulated the experience of the compound trials. A propositional account of conditioning, which considers the informative value of the CS to be the driver of conditioned responding[22, 31], may shed light on this. Vibrotactile stimuli (CSs here) are non-nociceptive and not inherently unpleasant or threatening, whereas laser stimuli are nociceptive and usually both unpleasant and threatening. It is plausible that the pairing procedure caused participants to learn (at some level) that the CS provides information about the 'dangerousness' of the laser stimulus specifically. If so, the modulatory effect of a CS may only occur when it is presented with a laser stimulus. In other words, the effect is on the perception of laser stimulus, with the CS playing the modulatory role, while the perception of the CS itself is unaffected. With this view, the valence and intensity of CS-only trials would not be altered, because the learning is specific to trials that involve both a CS and a laser stimulus.

That contingency awareness did not modulate the effect suggests a difference in perceptual experience[28] rather than response bias. However, it remains possible that participants may have been 'more ready' to report compound CS+/US_T trials as painful because of their experiences in the

acquisition phase. The relative contribution of response bias and altered perception is a ubiquitous problem when pain report is the primary outcome, but the key finding – that the association imparts the effect – remains important regardless of their relative contributions.

Implications

That persistent pain can be driven by classical conditioning mechanisms has long been suggested by clinical anecdote and by those who work with people in pain, but empirical support has been lacking. The current results lend preliminary support to the idea that classical conditioning may be an important role player in persistent pain and form a strong platform for pursuing this line of enquiry further. A logical extension will be to explore its relevance to clinical groups – most obviously, to profile aspects of associative learning in patients with sub-acute pain, so as to shed light on the possibility that conditioned shifts in pain threshold could underlie persistent pain that is not explained by tissue damage[23]. Previous work has demonstrated that people with chronic pain due to fibromyalgia, for example, learn contingencies more slowly, and learn about safety less thoroughly, than their healthy counterparts[21] (although people with chronic pain demonstrate other forms of cognitive impairment too[6, 7]). Whether these differences influence the development of chronic pain remains unclear. Replicating the present study in people with sub-acute or chronic pain could shed light on this issue.

A wider look at the field of classical conditioning and pain reveals a surprising paucity of studies[16]. Although nocebo research has found that adding a conditioning manipulation to verbal expectation boosts the hyperalgesic effect[27], studies of conditioning alone are few, and most measure hyperalgesia rather than changes at the pain threshold level. More research into the malleability of pain thresholds by classical conditioning would seem beneficial.

The idea that stimuli that are not inherently threatening (e.g. vibration, touch, movement) may come to elicit pain by a classical conditioning effect could provide a mechanistic explanation for

persistent pain after tissue healing[23] and prompt new treatments to prevent persistence. However, robust laboratory demonstrations of pain elicited by classical conditioning with an innocuous stimulus is clearly prerequisite.

Limitations

Our analysis of the intensity rating results treated the intensity FESTNRS as continuous, even though participants were forbidden from selecting zero on the scale. We took this approach because previous work showed robust properties of the FESTNRS and a strong linear relationship between laser stimulus intensity and FESTNRS ratings[15]. All questionnaires were, for practical reasons, completed before the procedure. It is possible that the assessment of state negative affect did not accurately reflect participants' affect during the procedure itself. Administering such questionnaires during mid-procedure breaks may remove this risk. Finally, our use of arbitrary noise to drown out equipment-related sounds may have decreased arousal levels, and arousal may be important for learning[10]. If so, however, this would have diminished learning, rendering the present results a conservative estimate of the classical conditioning effect.

Conclusion

We have shown that non-noxious stimuli that have been associated with painful nociception may later influence the perception of ambiguous nociceptive stimuli, such that those stimuli are perceived as painful, as more intense, and as more unpleasant. Our results show that this effect is unlikely to rely on a real-time change in fear, arousal or valence and imply that classical conditioning could be a useful framework for understanding persistent changes in pain threshold that are not explained by the state of bodily tissues.

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Figure legends

Figure 1: layout of vibrotactile stimulus probes and laser stimulation zones on the back.

Figure 2: The experimental procedure, comprising four phases, each including different quantities of each trial type. Dots denote vibrotactile stimuli, and darkened dots show which vibrotactile stimulus was delivered in each trial type. US = unconditioned stimulus.

Figure 3: The percentage of compound CS/UST trials (mean, SE) rated as painful by condition (CS+/CS-) and block (1, 2, or 3) of the test phase.

Descriptive data for sample (n = 16)		
Outcome	**Measure**	**Mean ± SD**
Age	Years	26 (range 18-61)
Positive state affect	PANAS (state)	27.31 ± 5.51
Negative state affect	PANAS (state)	11.53 ± 1.91
Depression	DASS subscale	4.44 ± 5.02
Anxiety	DASS subscale	3.44 ± 3.71
Stress	DASS subscale	9.19 ± 7.47
Pain catastrophising	PCS	16.31 ± 8.35

Table 1: Descriptive data for participants. PANAS: Positive and Negative Affect Schedule. DASS: Depression Anxiety Stress scale. PCS: Pain Catastrophising Scale.

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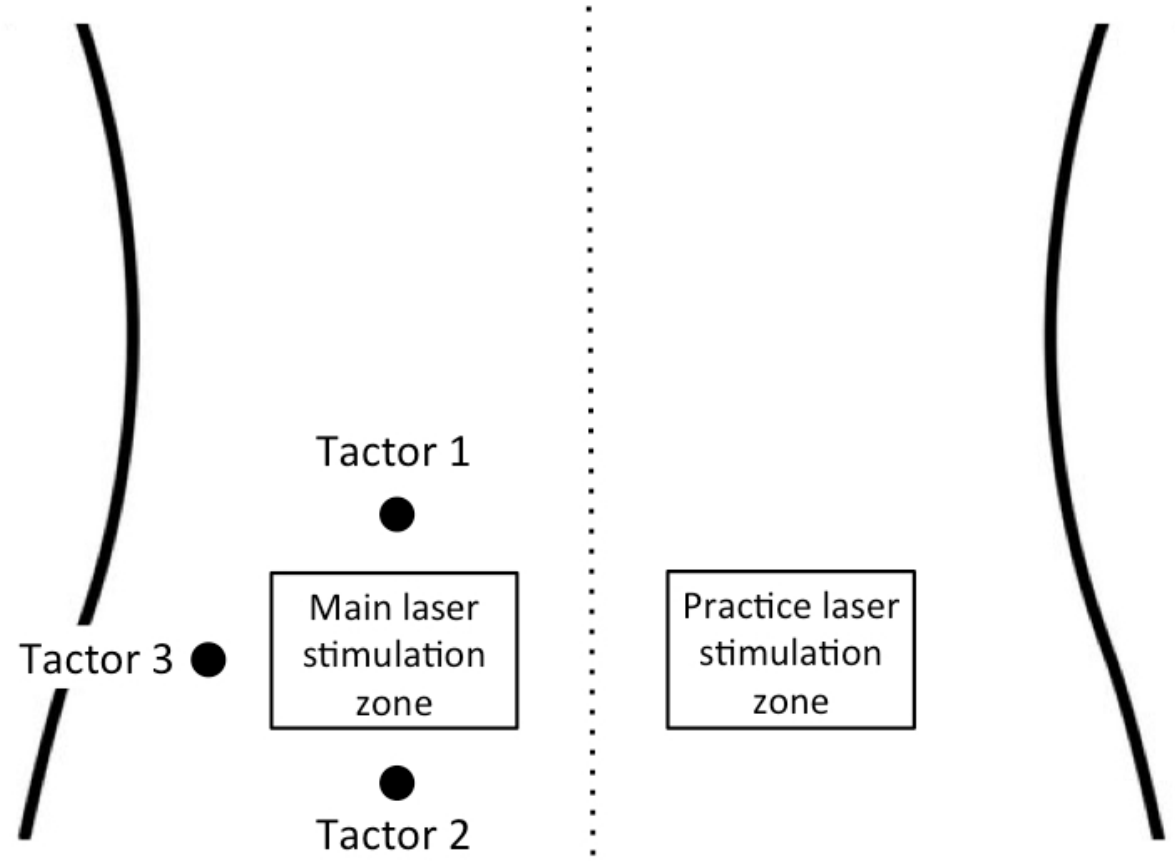
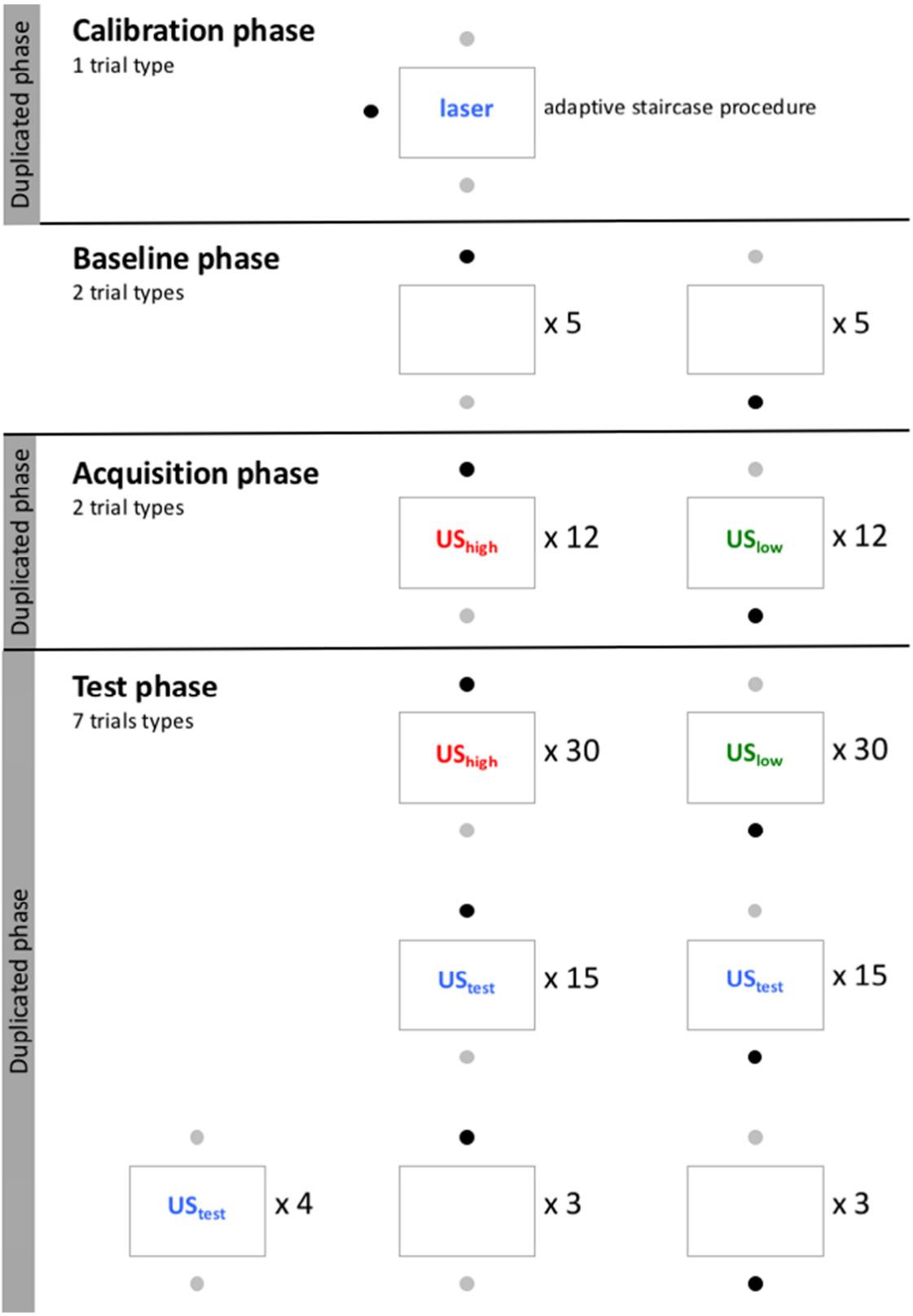
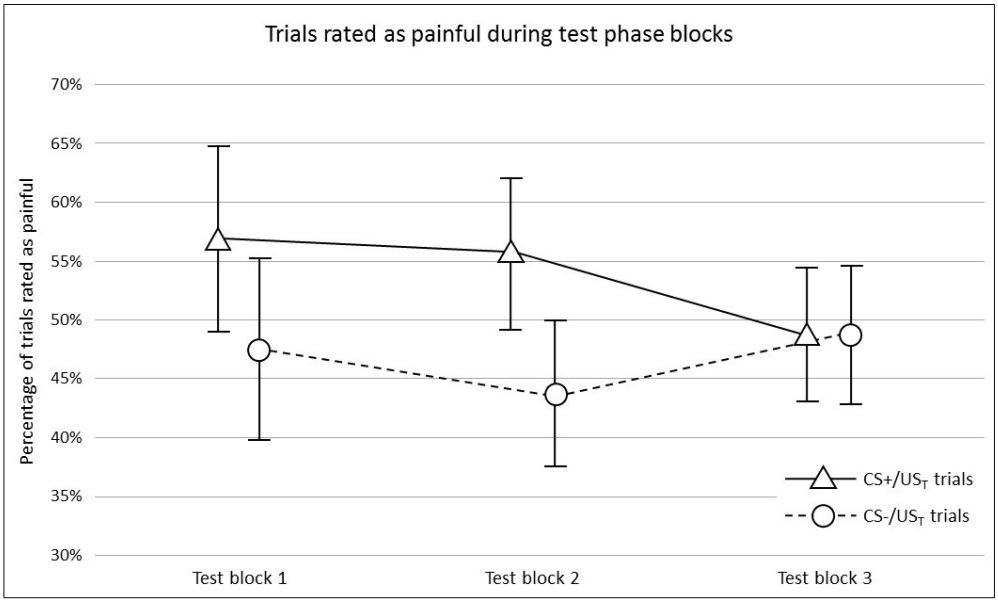
Total *N* = 16	Baseline		Acquisition		Test	
	Intensity	*Valence*	*Intensity*	*Valence*	*Intensity*	*Valence*
CS+ only	-30.66 ± 11.12	15.13 ± 13.04	-	-	-27.99 ±9.64	10.64 ±9.08
CS- only	-29.91 ± 11.81	15.49 ± 13.09	-	-	-28.62 ±9.97	10.75 ±9.15
CS+ with high-intensity laser stimulus	-	-	7.70 ± 6.20	-8.38 ± 6.93	7.99 ± 8.52	-9.78 ± 7.32
CS- with low-intensity laser stimulus	-	-	-13.89 ± 10.06	3.40 ± 4.43	-14.35 ± 10.48	3.01 ± 4.82
CS+ with at-threshold laser stimulus	-	-	-	-	-4.35 ± 9.90	-1.96 ± 3.46
CS- with at-threshold laser stimulus	-	-	-	-	-6.29 ± 9.98	-1.32 ± 3.39
At-threshold laser stimulus only	-	-	-	-	-12.98 ±18.38	-1.55 ±7.83

279 Table 2: Summary data (Mean ± SD) for ratings of intensity and valence on two FESTNRS scales, in each of the three phases.




Duplicated phase
Calibration phase
1 trial type
laser
adaptive staircase procedure
Baseline phase
2 trial types
x 5
x 5
Duplicated phase
Acquisition phase
2 trial types
US high
x 12
US low
x 12
Duplicated phase
Test phase
7 trials types
US high
x 30
US low
x 30
US test
x 15
US test
x 15
US test
x 4
x 3
x 3



Highlights

- Classical conditioning could drive pain to persist after tissue has healed.
- Neutral somatosensory stimuli could influence pain thresholds via conditioning.
- This study tested for conditioned alterations of human pain thresholds to laser.
- It used simultaneous pairing of non-noxious with noxious stimuli.
- The results showed a classically conditioned change in human pain thresholds.